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Precision and trueness verification of TSH, Ferritin and Vitamin B12 on Beckman Dxi 800

TSH, Ferritin ve Vitamin B12 testlerinin Beckman Dxi 800 cihazında kesinlik ve doğruluk verifikasyonu

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ABSTRACT

Objective: In medical laboratories, it is important to verify the precision and trueness studies made by the manufacturer before giving results to the patient. The aim of this study was to evaluate the verification of TSH, ferritin and vitamin B12 tests according to CLSI EP15 A2 guideline.

Methods: In this study verification studies were carried out according to CLSI EP15 A2 guidelines for TSH, vitamin B12, ferritin. PreciControl quality control material were used for precision study. For trueness studies, patient samples were simultaneously assayed with two different immunoassay analyzers (Beckman Coulter DXI 800 and Advia Centaur). Microsoft Excel programme was used for statistical evaluation.

Results: The precision estimate of TSH, vitamin B12, and ferritin for both control levels were lower than the verification value. In the trueness verification study, the estimated percent bias of TSH and vitamin B12 were within the verification limits. The estimated percent bias of ferritin was found beyond the verification limits.

ÖZET

Amaç: Tıbbi laboratuvarlarda, hastaya sonuç verilmeden önce üretici firma tarafından yapılmış olan kesinlik (presizyon) ve gerçeklik (trueness) çalışmalarının doğrulanması önemlidir. Bu çalışmanın amacı, hormon testlerinden TSH, vitamin B12 ve ferritin testlerinin CLSI EP15 A2 kılavuzuna göre verifikasyonunu değerlendirmektir.

Yöntem: Çalışmada TSH, vitamin B12, ferritin testleri için verifikasyon çalışmaları CLSI EP15 A2 kılavuzuna göre yapıldı. Kesinlik (presizyon) çalışmasında PreciControl kalite kontrol materyali kullanıldı. Gerçeklik çalışmasında ise hasta örnekleri iki farklı otoanalizörde (Beckman DXI800 ve Advia Centaur) çalışılarak hesaplamalar yapıldı. İstatistiksel değerlendirmeler için Microsoft Excel programı kullanıldı.

Bulgular: Kesinlik verifikasyon çalışmasında TSH, ferritin ve vitamin B12 testleri için hesaplanan değerler verifikasyon değerlerinin altındaydı. Doğruluk çalışmasında ise TSH ve vitamin B12 testlerinin hesaplanan bias değerleri verifikasyon limitleri içerisinde iken, ferritin testinin ise hesaplanan bias değerlerinin verifikasyon limitlerinin dışında olduğu gözlendi.

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Conclusion: We found the precision and trueness of Vitamin B12 and TSH measurement methods acceptable in our study, we showed that the precision of the ferritin measurement method was acceptable but its trueness had an unacceptable negative bias.

Key Words: TSH, Vitamin B12, Ferritin, EP15 A2, method verification, precision, accuracy

Sonuç: Çalışmamızda Vitamin B12 ve TSH ölçüm metot performansları kabul edilebilir limitler içerisindedir, ferritin kesinlik çalışması kabul edilebilir limitlerde olmasıyla birlikte doğruluk çalışmasında negatif bias gözlenmiştir.

Anahtar Kelimeler: TSH, Vitamin B12, Ferritin, EP15 A2, metot verifikasyonu, presizyon, gerçeklik

INTRODUCTION

Method validation is a process that determines the performance characteristics of clinical laboratory testing measurement procedures (1). According to The International Vocabulary of Metrology (VIM) 3, verification is "provision of objective evidence that a given item fulfils specified requirements" (2). The majority of measurement methods used in laboratory is validated by diagnostic companies (3). Validation is often done in ideal laboratory conditions. However, the performance of the method validated by the manufacturer may not be appropriate for the expected targets due to the differences in conditions in clinical laboratory practice. Bioanalytical verification procedures test whether the performance data obtained by manufacturers during method validation can be reproduced in the environment of an end-user (4).

Clinical laboratories have an important role in patients' admission, discharge and treatment (5). In this context, it is important for both clinicians and patients that laboratory results provide the expected performance. The quality of laboratory services directly affects the quality of health services (6).

Thyroid-stimulating hormone (TSH) is a hormone produced by the thyrotrope cells in the anterior pituitary glands and an important test for diagnosing and managing various thyroid gland disorders. Evaluation of serum TSH concentration is critical in the diagnosis and treatment of thyroid diseases. It is the best screening tool for thyroid dysfunction. (7). Vitamin B12 is a water-soluble vitamin and needed for cell maturation and DNA replication. Vitamin B12 deficiency can cause megaloblastic anemia and neurological problems (8). Ferritin is an important iron storage protein and for iron homeostasis. It plays a role in various physiological and pathological processes. In clinical medicine, it is mainly used as a serum marker of total body iron stores and reflects iron status of individuals (9). The aim of study is to evaluate the imprecision and bias verification of TSH, ferritin and vitamin B12 measurement procedures according to the clinical & laboratory standards institute (CLSI) guideline EP15-A2 (User verification of performance for precision and trueness; approved guideline, 2rd edition) in Beckman DXI 800 autoanalyzer.

MATERIAL and METHOD

UniCel DxI 800 is a random access Beckman Coulter immunoassay system. It uses magnetic particle separation and indirect chemiluminescent technology. Measurement principle is Dioxetane-based chemiluminescent (Lumi-Phos*) method. The system processes up to 400 tests per hour.

The study was performed in the clinical chemistry laboratory of Izmir Su Hospital in March 2019. Method verification of TSH, ferritin and vitamin B12 tests were performed according to CLSI guideline EP15-A2 (10). TSH, ferritin and vitamin B12 tests were analysed on both Beckman Coulter Unicell DXI 800 and Advia

Centaur immunoassay analyser.

Verification of precision performance

İnternal quality control materials were used to evaluate verification of imprecision. Precicontrol 1 and precicontrol 2 were analyzed one run day with three replicate samples at each of two concentrations daily for five days (15 replicates per level). Run data that had to be rejected due to quality control procedures or operational difficulties were discarded and an additional run was performed. The data were collected and transcribed onto an appropriate recording sheet.

Repeatability (within run precision) and within laboratory precision were calculated with formulas according to EP15 - A2. The verification value was calculated when the standard deviation was calculated higher than the standard deviation declared by the manufacturer (σr: manufacturer's declared SD for repeatability, σ1: manufacturer's declared SD for within laboratory imprecision).

Repeatability (within run precision): The degree of freedom (V) was calculated as 10 by the formula V=D*(n-1) for five days (D) and three replicates (n). Percentage point (C) of the chi-square distribution [l- The false rejection rate (α)/ the number of concentration levels tested (ℓ)] was determined as 20.48 according to the degree of freedom. Verification value was calculated using verification value=(σr*√C)/√v formula.

Within laboratory precision: The degree of freedom (T) was determined for five days and three replicates (n). T values were calculated according to CLSI guideline EP15-A2. C value of the chi-square distribution (1- α/ℓ) was determined according to the degree of freedom. Verification value was calculated using verification value=(σ_1*√C)/T formula.

Demonstration of Trueness

Patient sample results were compered to another measurement procedure. 20 patient samples were used for trueness study. Samples whose concentrations did not exceed the measuring range of the measurement procedure were included in the study. Appropriate routine quality control procedures were followed for each measurement procedure. Samples were analyzed within the same run on Advia Centaur and UniCel DxI 800 immunoassay analyzer. The measurements were completed within four hours on the same day.

Trueness estimation was determined by comparing %Bias obtained from patient samples against B (desirable specification for inaccuracy) (7). The difference between each sample result (b_i) for the procedures was calculated. Bias estimation was determined using

$\%b_i$=100*(test procedure $result_i$-comparison procedure $result_i$)/comparison procedure $result_i$ formula.

Verification limits for percent bias calculated according to the formulas Ƀ-($t*s_{(\%b)}$)/√n and Ƀ+($t*s_{(\%b)}$)/√n. Standard deviations of bias (s%b) in percent were calculated according to CLSI guideline EP15-A2. The (100- α) percent point of the t-distribution with n-1 (n= the number of patient samples) degrees of freedom (t) determined as 2,539. The degree of freedom and α value was 19 and %1 respectively.

RESULTS

Repeatability (within run precision) and within laboratory precision study data of TSH, vitamin B12, ferritin measurement procedure were shown in table 1. The manufacturers claimed target values, calculated repeatability (within run precision), within laboratory precision, and verification values data are presented in Table 2.

For both control levels, the estimated repeatability (within run precision) values of TSH, vitamins B12, and ferritin were lower than the verification value.

The within laboratory precision estimate of TSH, vitamins B12, and ferritin for both control levels were lower than the verification value (Table 2).

In the trueness verification study, the estimated percent bias of TSH and vitamin B12 were within the verification limits. The estimated percent bias of ferritin was found beyond the verification limits (Table 3).

Table 1. Repeatability and within laboratory precision experiment data of TSH, vitamin B12 and ferritin measurement procedures

TSH					
Precicontrol 1	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**
Replicate 1	2.84	2.91	2.78	2.81	2.75
Replicate 2	2.8	2.9	2.88	2.75	2.78
Replicate 3	2.8	2.88	2.9	2.83	2.83
Precicontrol 2	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**
Replicate 1	10.11	10.22	10.52	10.1	10.3
Replicate 2	10.09	10.11	10.41	10.05	10.22
Replicate 3	10	10.05	10.39	10.01	10.4
Vitamin B12					
Precicontrol 1	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**
Replicate 1	284	309	292	305	301
Replicate 2	290	300	295	300	298
Replicate 3	274	301	300	293	290
Precicontrol 2	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**
Replicate 1	549	536	533	530	550
Replicate 2	560	555	558	548	545
Replicate 3	545	543	545	535	538
Ferritin					
Precicontrol 1	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**
Replicate 1	70.5	70.6	68.8	69.5	68
Replicate 2	70	71.9	68.3	69.9	67.2
Replicate 3	70.2	73	69	70	69
Precicontrol 2	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**
Replicate 1	444.6	484	501.5	442.6	471
Replicate 2	442	484.5	500	444	472
Replicate 3	441.9	489	490.5	445.5	475.4

Table 2. The manufacturer's claimed target values, calculated repeatability (within run precision), within laboratory precision, and verification values for TSH, vitamin B12 and ferritin measurement procedures

Tests	**IQC**	**S_r**	**Manufacturer's claimed S_r**	**Verification value S_r**	**S_1**	**Manufacturer's claimed S_1**	**Verification value S_1**
TSH	PCC1	0.041	0.03	0.043	0.056	0.24	0.252
	PCC2	0.072	0.17	0.243	0.179	0.24	0.219
Vitamin B12	PCC1	5.91	17.9	25.616	9.146	24.6	40.793
	PCC2	9.29	53.4	76.42	9.003	58.12	87.998
Ferritin	PCC1	0.71	0.96	1.374	1.57	1.52	2.364
	PCC2	3.25	12.16	17.402	24.68	19.6	32.708

(IQC: Internal quality control, PCC: Precicontrol, S_r: Within run precision, S_1: Within laboratory precision)

Table 3. Desirable specification for inaccuracy, bias and verification limits of TSH, vitamin B12 and ferritin measurement procedures

Tests	Desirable specification for inaccuracy	Bias (%)	Verification limits
TSH	7.8	-16.20	(-1.40) - (-17)
Vitamin B12	17.7	-15.74	8.76 - (-26.64)
Ferritin	5.2	-17.59	(-4.79) - (-15.19)

DISCUSSION

Verification of measurement methods are procedures that provide end-users with confidence that measurement methods are suitable for their intended purpose. In the method selection process, laboratories should first determine the required performance according to the legal requirements and/or the medical usefulness requirements of the method. After, a method that claims to meet the desired performance characteristics should be chosen. The laboratories should verify that the manufacturer's claimed imprecision and bias are achieved by their laboratory by the laboratory.

We conducted an imprecision and bias verification experiment for the vitamin B12, TSH, and ferritin measurement procedure performed on the Beckman DXI800 analyzer, which we started to use as a new system in our laboratory. We determined that the estimated repeatability and within laboratory precision values of TSH, vitamin B12 and ferritin tests were lower than the calculated verification values. According to CLSI guideline EP15-A2, if the estimated repeatability values are less than or equal to the verification value, data is accepted consistent with the manufacturers' claim for repeatability, and the claim is verified. Based on the data we obtained, we verified the precision performance characteristics of TSH, vitamin B12 and ferritin measurement methods.

The total error consists of random and systematic components. The random error is described qualitatively as precision and can be measured quantitatively as SD. Systematic components are described qualitatively as accuracy and measured quantitatively as bias (4).

The choice of the reference measurement procedure in the accuracy study is important for the interpretation of the experimental results. If the new procedure is not a revision of the same manufacturer's previous procedure or the application of the manufacturer's previous procedure to a new device, it is not appropriate for the laboratory to demonstrate the trueness with the manufacturer's claim. If the trueness experiment is performed using a different measurement procedure, it is recommended to determine a medically allowable bias between the results obtained with the new procedure and the comparative procedure. The determined bias should use as the basis for demonstrating the trueness. The measurement procedure we compared in our trueness experiment was different from the measurement procedure we used. We determined the verification limits using the desired biological variation values for TSH, ferritin, and B12, rather than the bias value declared by the manufacturer. We think that evaluating the trueness between different procedures ensures us to evaluate the relationship between the procedures and the ability of our laboratory to produce equivalent results with different procedures (10).

The estimated percent bias values of TSH and B12 tests were within the verification limits we calculated. We have thus confirmed that the TSH and B12 measurement procedures demonstrate a percent bias consistent with the manufacturer's claim. We determined that the estimated percent bias value of the ferritin test was beyond the verification limit. Test results of the ferritin measurement method in Beckman DXI were lower than that of Siemens Advia Centaur.

Kristensen et al. evaluated four immunological test systems in their study, including Beckman DXI and Siemens Advia Centaur. The bias of the ferritin measurement procedure was found beyond the manufacturer's target bias. The bias of the ferritin measurement method was determined as -13.8% for the Beckman Unicel automatic analyzer and -8.7% for the Siemens Advia Centaur. In the evaluation made in the study, the bias obtained for both measurement methods were beyond the desired bias limits (11). According to the CLSI guideline EP15-A2, if it is detected that the estimated percent bias is outside the verification limits, it is recommended to contact the manufacturer for assistance (10). In this direction, we contacted the manufacturer and planned to repeat the study after suggestions and necessary interventions were made.

As a result, while we found the precision and trueness of vitamin B12 and TSH measurement methods acceptable in our study, we showed that the precision of the ferritin measurement method was acceptable but its trueness had an unacceptable negative bias. Our study shows analytical verification results for commonly used immunoassay tests that are clinically important for Beckman DXI800. We believe that the results of our study and the approaches we take based on the results we have obtained will be useful to laboratory managers.

ETHICS COMITTEE APPROVAL

* This study does not require Ethics Comittee Approval.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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